Animal Models for the NTP Rodent Cancer Bioassay: Strains & Stocks -- Should We Switch?

The NTP held this workshop on June 16 and 17, 2005, at the NIEHS in Research Triangle Park, NC as part of an activity of the NTP Roadmap to critically review its testing program and determine whether any refinements are needed in the protocols. Approximately 100 attended with international and national representations including invited participants from NTP-participating regulatory and science agencies, external NTP advisory groups, industry, academia, animal welfare groups, and contract companies.

Objectives. The workshop's objectives were: to determine (1) whether the currently used models, the F344/N rat and BC3F1/N mouse, continue to be appropriate to identify substances that may pose a carcinogenic hazard for humans and (2) whether the NTP should consider conducting cancer bioassays using multiple strains of rats and/or mice to better capture the range of genetic variability.

Format. The workshop included plenary talks outlining NTP's issues with the current models, perspectives from a pharmaceutical company and a research organization on model selection, and an overview of the multiple strain approach including a presentation of statistical power. The attendees were then divided among three breakout groups to discuss the issues noted above. The attendees ultimately reconvened in plenary session on the second day to discuss the deliberations of the individual breakout groups. Background materials, the list of workshop participants, and presentations can be found on the NTP website (*http://ntp.niehs.nih.gov* see "Meetings & Workshop").

Findings from the workshop were presented to the NTP Board of Scientific Counselors at its meeting on August 18, 2005. The following text summarizes the workshop's recommendations and discussion by the NTP Board and outlines NTP's next steps with regard to strains and stocks for its rodent cancer bioassay.

Workshop Recommendations, Board Discussions, and NTP next steps:
F344/N rat: The rat breakout group strongly advised the NTP to discontinue using the current F344/N strain due to the recent appearance of problems with fertility, seizure activity, and chylothorax. The breakout group suggested three options: (1) re-establish the F344/N strain, although such an approach would not address the general issues confronting the strain (e.g., relatively high background rates of testicular interstitial cell tumors and mononuclear cell leukemia); (2) create an F1 hybrid, such as the F344/Brown Norway cross (FBNF1) often used in aging studies due to its longevity; or (3) consider using an alternative strain or stock such as the outbred Wistar Han. The Wistar-Han is one outbred strain used by several pharmaceutical companies because of its long survival and low background tumor incidence.

With respect to how NTP should switch strains, the group said the NTP should complete studies already initiated with the F344/N. For future bioassays, the group recommended that the NTP only use the new strain without concurrent testing in the F344/N. The group felt that the new strain could be considered the default unless metabolism data suggested otherwise.

The Board concurred with the workshop recommendations. One member noted that the Brown Norway rat has a low background incidence of mononuclear cell leukemia and when crossed with the F344 strain, the F_1 has a lower incidence of leukemia than the parental F344 strain.

The NTP discontinued use of the NTP F344/N in all its toxicity studies shortly after the workshop. Beginning in October 2005, the NTP began using a commercial F344 from Taconic Farms Inc. (F344/NTac). The first study with this strain is an acute inhalation study on sandblasting particles. The NTP intends to continue to use an isogenic rat strain in order to maximize the animals' reproducibility of response over time to exposed substances and facilitate genetic monitoring and interpretation of subsequent mechanistic studies. The NTP is working with Taconic Farms, Inc, to establish a new line of F344 that is expected to be ready in mid-2006. In addition, the NTP may explore the FBNF1 as an alternative strain. While the FBNF1 lacks a historical control database to assess spontaneous neoplastic and non-neoplastic lesions, the NTP could establish a control database by using the FBNF1 as an additional concurrent control in chronic studies. If needed, testing the FBNF1 with a known carcinogen and then comparing the results to the animal strain used to identify the substance initially as a carcinogen could be done to validate the sensitivity of the FBNF1 to carcinogens

<u>B6C3F1/N mouse</u>:
The mouse breakout group did not believe that the issue of a high background liver tumor incidence is currently critical enough to merit a switch in mouse models. However, they strongly suggested that the NTP have the DNA of the parent strains of the B6C3F1 sequenced to gain a better understanding of the genetic makeup of the model. The mouse breakout group thought that it is very important to understand the basis for the purported lower liver tumor background incidence in the NCTR B6C3F1 mice.

The Board agreed that the NTP should not change the $B6C3F_1$ mouse strain. The strain used at NIEHS is heavier than the strain used by NCTR scientists and the Board suggested that it might be useful to compare the genetic make-up of the two strains.

At this time the NTP has no plans to switch mouse models although the NTP will consider the use of alternative mouse strains as the NTP explores the multiple strain approach. The DNA of the parent strains of the B6C3F1 model is being sequenced.

<u>Multiple Strain Approach</u>:
In brief, a multiple strain approach involves the use of more than one strain of rat or mouse in each dose group. The total group size per dose would be comparable to the current single strain approach. For example, if 5 strains of mice were used, each dose group could be comprised of 10 animals per strain for a total 50 animals per dose per sex.

From a research perspective, the use of multiple rodent strains in the bioassay may increase the NTP's understanding of genetic susceptibilities to environmental exposures and improve our ability to extrapolate findings to humans. For these reasons, the draft NIEHS strategic plan for 2006 highlights the use of a variety of rodent strains to improve the availability of relevant *in vivo* models for human disease. Complementary to this objective is a research plan initiated in 2004 by the NIEHS and NTP for whole genome DNA sequencing of fifteen inbred mouse strains.

While there is considerable support for the multiple strain approach as a research tool, its utility for hazard identification purposes is uncertain. Based on simulations using 1, 2, 3, or 4 strains, statistical power to detect a carcinogenic response is generally similar between the multiple and single strain approaches except in situations where there is a considerable amount of heterogeneity in tumor response *and* the most sensitive strains display a "very strong" response. In this case, the multiple strain approach may increase power by up to 45 to 70 percent, depending upon the number of strains used. However, any theoretical power advantage of a multiple strain approach is lost if each strain is analyzed separately ("multiple separate test") instead of pooling across strains ("pooled test"). This issue is potentially a major hurdle for routine use of the multiple strain approach as it is unclear whether regulatory and scientific communities would accept the results of a pooled analysis.

The multiple strain breakout group had considerable discussion about whether the NTP should conduct chronic toxicity/carcinogenicity bioassays in multiple strains of isogenic rodents to better capture the range of genetic variability. Specifically the group suggested that the NTP have a repository of possible strains and choose the best model(s) to use in the bioassay based upon knowledge of proposed targets for toxicity. Overall, they thought it is a viable approach for cancer hazard identification[1] and discussed some of the advantages and disadvantages. However, the multiple strain group did not attempt to weigh the advantages and disadvantages to provide an overall recommendation on whether the NTP should routinely adopt the multiple strain approach. The cost and complexity of conducting chronic assays in multiple strains are major factors that need consideration.

The Board expressed mixed opinions on the multiple strain approach (see attached August 18, 2005 Board minutes) and ultimately suggested that the NTP present the Board a proposal on its priorities for the testing program to which the Board could respond.

The NTP is deferring a final decision on whether/how to pursue the multiple strain approach until other workshops related to the NTP Vision and Roadmap are completed. At that time, the program will reevaluate priorities related to the testing program and consider broader issues relating to an increased emphasis at the NIEHS toward addressing host susceptibility.

[1] The rat breakout group was not supportive of a multiple strain approach that involves keeping group sizes the same as the current study design (n = 50 per sex per group), but composed of animals from different strains (e.g., 10 animals per sex from 5 strains). Primarily, they were concerned about interpreting a study based on pooling data from small groups of animals from different strains. For this reason, they felt the multiple strain approach would not be practical as it would have to be scaled up appropriately to mimic a single strain study design.